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REVIEW OF THE WORK OF THE LABORATORY OF M. I. VOLSKIY "ON THE ASSIMILATION OF ATMOSPHERIC NITROGEN BY HIGHER ORGANISMS"

Ye. N. Mishustin

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Ye. N. Mishustin

ABSTRACT: The article represents a critical review of works published by the M. I. Volskiy Laboratory and by others on the assimilation of nitrogen by higher organisms - man, animals and plants. M. I. Volskiy tries to prove that all organisms fix molecular nitrogen, regardless of symbiosis with microorganisms. Critical analysis reveals, however, that such statement is unfounded. Volskiy's experiments were not reproducible. M. I. Volskiy does not use the literature objectively and ignores materials that are contradictory to his views. Existing evidence proves that only lower life forms are capable of fixing nitrogen.

INTRODUCTION

M. I. Volskiy's Publications (1961 - 1970) concerning the fixation of /647* molecular nitrogen by higher plants and animals, attracted world wide attention, particularly after the appearance in several newspapers and journals of reports on research data. Articles on M. I. Volskiy's work have been printed not only in the U.S.S.R., but abroad as well.

Reports on M. I. Volskiy's work were published basically under the title "Azotom Dyshit Vse Zhivoye" as translated "All Living Things Breathe Nitrogen" it was confirmed that the previous unknown capability of animals and plants to assimilate atmospheric nitrogen required for normal vital activity, has been established.

The author of the present article familiarized himself with the printed works of M. I. Volskiy and his co-workers. The opportunity arose to visit the M. I. Volskiy laboratory and to discuss the work with staff members. All this led to certain conclusions, with which the author of the present work would

* Numbers in the margin indicate pagination in the foreign text.

like to familiarize the scientific community so that it can become aware of the scientific significance of M. I. Volskiy's works.

In undertaking the solution of the stated problem, we consider it useful first of all to review briefly the current state of the question at hand.

That molecular nitrogen is fixed by a rather large group of microorganisms can be considered proved. Some of these microorganisms live freely in the environment (in the soil or water), and others have symbiotic relations with higher organisms.

The free-living nitrogen fixers include aerobic and anaerobic bacteria, blue-green algae and other microorganisms (Mishustin, Shil'nikova, 1968).

There is a large group of leguminous and non-leguminous plants that symbiotically fix molecular nitrogen. All symbiotic nitrogen fixers have special formations ("tubercles"), most often in the roots, and sometimes on the leaves. These tubercles are populated by microorganisms. N_2 is not bonded in the absence of microorganisms.

It was shown later by rather old verified tests that N_2 can be fixed by insects. This was established by the Czechoslovak researcher Peklo (1946), and by the Hungarian scientist Toth and co-workers (1946). Insects fix nitrogen by means of nitrogen fixing microorganisms that populate special organs in great masses.

That molecular nitrogen is unimportant in the nitrogen balance of higher animals and man is considered firmly established. Of course variously nitrogen fixing bacteria are found to come along with others, in the intestines and internal organs of animals. This is not surprising, since it is generally known that microorganisms populate the intestinal tract, once individual organisms invade the internal organs and blood. There may be among them nitrogen fixing bacteria which are widespread in nature (Polonskaya et al., 1962, 1963; Orobinskiy, 1960, 1962; Bergersen, 1970; Postgate, 1970, among others).

It must also be remembered, however, that nitrogen fixation by microbes takes place in a nitrogen impoverished environment, since bonded nitrogen is substantially easier for them to assimilate than molecular nitrogen. The internal environment of the higher animals and man is unfavorable for the assimilation of N_2 by microbes. A possible exception is the rumen of ruminants where microbiological processes proceed vigorously and protein, in particular, can be synthesized from mineral compounds of nitrogen. In the case of poor feeding with a low concentration of nitrogen compounds, nitrogen assimilation may occur here as a result of bacterial activity. This process, however, has hardly significant importance. Otherwise there would be no worry about supplementing animal feeds with nitrogen, which is of such great concern to agricultural workers. We may recall that when feed is protein deficient, two to three times more feed than normal is consumed.

Protein deficiency as well as deficiency of the essential amino acids, in the human diet has serious consequences. Moreover bacterial nitrogen fixers are found in the intestines of man, which are capable of bonding some molecular nitrogen when the human diet is rich in carbohydrates and poor in protein. According to all data, however, their activity in human nitrogen metabolism in view of the above, is of minor importance.

Thus, all existing data indicates that only lower life forms are capable of bonding molecular nitrogen. When microbes exist in clear symbiosis with higher organisms, the assimilated molecular nitrogen can be used by the later. In this case, of course, massive multiplication of the microorganisms is required within the higher symbiotic partner where they usually populate special organs.

M. I. Volskiy attempts to prove that all organisms, regardless of their relation with microorganisms, are capable of fixing molecular nitrogen. This assumption should in principle be considered completely possible. Obviously, the first organisms that inhabited the earth developed in the absence of oxygen and probably assimilated molecular nitrogen (Oparin, 1967). We may assume that this capability remained to a greater or lesser extent in modern higher life forms, if only as a "physiological appendix". Nitrogen fixing activity should be most clearly manifested in the embryonic period of development. Therefore, M. I. Volskiy's effort to check his assumption may be considered completely founded.

It is noteworthy that such attempts to expand the community of organisms capable of fixing molecular nitrogen, were undertaken long ago. Thus, in his classical work "Svyazyvaniye Atmosfernogo Azota Pochvennymi Mikrobami" translated Bonding of Atmospheric Nitrogen by Soil Microbes" V. L. Omel'yanskiy (1923) writes a long list of studies aimed at proving that practically all plants can assimilate atmospheric nitrogen without the aid of microorganisms. A careful check of these works revealed errors.

Recently, in the U.S.S.R., F. V. Turchin el al. (1963) using what seemed to be completely modern procedures in tests with N_2^{15}, attempted to restore the abandoned concepts. However, errors were also found in his experiments (see below).

Thus, existing experience compels great diligence and scientific circumspection in studies on the problem of interest to us.

Biochemistry of Bonding of Molecular Nitrogen in M. I. Volskiy's Concept

Molecular nitrogen is extremely inert, and its assimilation by microorganisms becomes possible in view of the presence in them of the ferment of nitrogenase which activates both into and the hydrogen donor system.

There is voluminous literature on the subject of nitrogenase and mechanism of nitrogen fixation process. Much of it is found in monographs by Ye. N. Mishustin and V. K. Shil'nikova (1968), also by V. I. Lyubinov (1969).

The above mentioned investigations point out the complexity and the multi--staged character of the reduction of N_2 to NH_3, and the formation of nitrogen organic compounds. The general scheme of the catalytic process is discussed.

M. I. Volskiy, who adheres to his unique concepts which are very far from the contemporary ones, did not make complete use of all this voluminous literature. According to M. I. Volskiy, atmospheric nitrogen can be assimilated by plants, animals and man in the following ways:

1- By keto- and amino acids that exist in the organisms; 2- by aeroions that act on higher organisms; 3- by trace elements (Li, Fe, Mo, and so on); 4- under the influence of bioelectricity occuring in the organism; 5- with the participation of bacteria that inhabit the organs of plants and animals.

The first of the above listed methods was discovered by V. S. Sadikov (1917) who considered bonding not only possible by keto- and amino acids, but also by the hydrolizates of proteins. This assertion is scientifically unfounded and contradicts the wealth of experimental data that has now been accumulated.

The position that the bioelectricity of organisms and aeroions are capable of activating the N_2 molecule can also be considered unfounded in view of their natural activity.

Trace elements do not exist in the body in the free state period they enter /649 only into catalytic systems.

The assimilation of N_2 by higher organisms by means of bacteria is a reality. We have already discussed this above, and it has long been known. However, M. I. Volskiy's assertion that bacteria that populate the tracheal passages of animals and man play an important role in nitrogen fixation, is completely unfounded. Massive populations of bacteria do not occur here, and singular microorganisms cannot play a notable role in nitrogen metabolism.

As a whole, the scientific foundation upon which M. I. Volskiy constructs his theoretical work is far below the contemporary level.

Assimilation of Molecular Nitrogen by the Human Body According to M. I. Volskiy

M. I. Volskiy states that the human body and animals assimilate a rather large quantity of molecular nitrogen. This conclusion lacks experimental confirmation and is based on certain basically very out-dated materials (Sechenov, 1873), according to which arterial blood contains more nitrogen than venous. New data (Saunders, 1958) do not establish such a difference in the composition of the gases of arterial and venus blood of man and animals.

M. I. Volskiy, arbitrarily using the material that suits him, figures that the human body assimilates an average of 26.5 grams of molecular nitrogen daily (Volskiy, 1961, p. 370), from which may be synthesized 165.6 grams of protein, quantity that completely satisfies man's protein requirement, at least for a day and a half. If this were true, however, man, like animals, would never experience protein deficiency. Thus, experience contradicts M. I. Volskiy's conclusions.

We will assume however that M. I. Volskiy is correct and that microorganisms in the human body (those that can bond in two) convert gaseous nitrogen to protein

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It has been established that bacteria require at least one gram of sugar or the equivalent amount of other carbon compounds to bond 10-15 milograms N_2. It is easy to calculate that the bacteria consume at least 2.2 kilogram of sugar daily, i.e. the amount of carbohydrates consumed by the human body in at least 4-5 days (!), to assimilate 26.5 grams of nitrogen. The question arises: How, then, are other functions of the human body guaranteed?

M. I. Volskiy, without any experimental data, expresses the opinion that N_2^{15} is easier to get into the protien molecule than N_2^{14}. (He calls N_2^{15} "unsupplemented"). We know, however, that the difference in the rates at which the isotopic molecules react is determined in the first approximation but the square root of their mass ratio. Calculations show that this difference in the case of N_2^{15} is about 7%, i.e. minor. Meanwhile, M. I. Volskiy recommends that space ship cabins be enriched with the isotope N_2^{15} and he writes: "It is our deep conviction that we should be concerned in space flights about providing the test animals not only with oxygen but also with nitrogen...we recommend that the cabin of satellites be enriched with the isotope N_2^{15}, since it is more readily assimilated by the organism than the isotope N_2^{14}" (Volskiy, 1961 p. 437). This is a characteristic example of the extremely peculiar attitude that M. I. Volskiy takes toward the important conclusions that the reader will encounter when reading his works.

If indeed N_2^{15} were more readily assimilated than N_2^{14}, the protien of man and animals would be greatly enriched with the heavy isotope. Such a fact has never been recorded, and M. I. Volskiy, in his many years of work with the assimilation of molecular nitrogen by animals, has never given a single determination of N_2^{15} in man or animal, however, that might confirm his point of view.

Thus, M. I. Volskiy's assertion about the assimilation of N_2 by man and animals is not based on reliable information. On the contrary, both certain literature data and experiments of the M. I. Volskiy laboratory speak of the possibility of a diametrically opposed process--separation of free nitrogen from decomposing organic compounds, takes place in higher life forms.

Literature Data on Fixation of Molecular Nitrogen by Man and Mammals

In presenting his views, M. I. Volskiy states that they are confirmed both by Soviet and foreign investigators. We will attempt to analyze this, using the literature references of M. I. Volskiy and literature with which we are familiar.

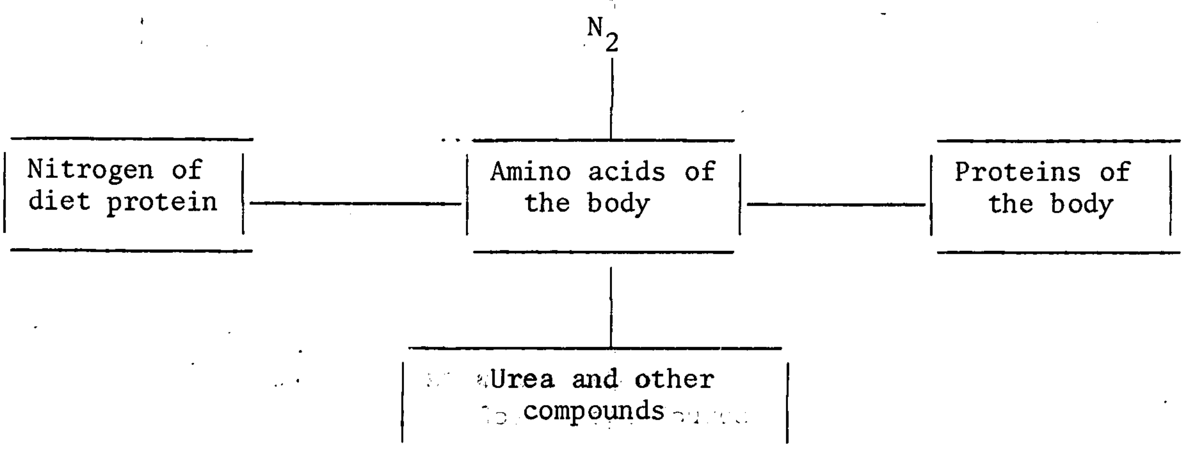
M. I. Volskiy (1970, p. 4) states that the foreign investigators Scano and Costa verified his data on the assimilation of N_2 by higher animals.

Scano's materials (1958), give no information on the fixation of N_2 by animals. He discusses only the influence of various gas mixtures on the developing embryos of birds (see below).

M. I. Volskiy's reference to Costa's data is based on a letter that this /650
scientist wrote to him in 1962 (Volskiy, 1970, p.85). In a translation of the letter, done at the M. I. Volskiy laboratory, is the sentence: "I repeated at least part of your experiment on eggs and found that assimilation of nitrogen *obviously* (the italics are mine.- Ye. M.) takes place during the incubation process..."

Costa, as follows from the letter, conducted his test only with eggs, but did not work with mammals. Errors were made in the translation. Costa writes: "There seems to be an accumulation of N_2...", i.e. "...kak budto by ineyetsya usvoyeniye azota..." now as translated there seems to be some assimilation of nitrogen. Thus, Costa uses more than careful formulation, far from confirming the disputed fact.

I felt it useful to write Dr. Costa and ask about his attitude toward the question brought up by M. I. Volskiy. In his reply (January 1971), Costa reported to me that he tried in vain to establish scientific contact with M. I. Volskiy. Costa remarked that at that time he had no data on which to conclude that the process of nitrogen assimilation occurs in mammals (verbatum "we have no evidence thus far that N_2 can be assimilated by mammals"). Costa makes absolutely no reference to his test with eggs. Hence it should be concluded that they did not yield positive results, since otherwise Costa would have been able in the last nine years to publish a sensational work, just as he published other studies, to which M. I. Volskiy never refers. Meanwhile, in recent years Costa and his co-workers (1960-1968) published several works on nitrogen metabolism of man and animals which do not speak in behalf of M. I. Volskiy's fuse of assimilation of molecular nitrogen by higher life forms. On the basis of experiments these investigators concluded that during transformation of organic compounds in the human body and in animals, nitrogen is partially lost in the form of N_2. The new hypothesis is represented by the following diagram:



We might point out that even M. I. Volskiy noted the possibility of such a process (1961, PP. 155-158), but he lacked experimental data and he reached his conclusions through calculations.

M. I. Volskiy cites work by Muysers as verification of the fixation of molecular nitrogen by higher animals. The fact is that Muysers (1969) is inclined toward Costa's views and speaks not of the assimilation of molecular nitrogen by higher organisms, but of the diametrically process--liberation of free nitrogen by them. M. I. Volskiy and his co-workers deviate from careful analysis of experimental data obtained by Muysers. Dr. Muysers, in reply to our written request, reported (1971) that he could not establish the fact of fixation of N_2 by higher organisms. He wrote: "

The views of Costa and Muysers may be disputed, but in any case their data does not support M. I. Volskiy's theory of nitrogen fixation by man and higher animals. On the contrary, they indicate the diametrically opposed process.

An article by R. Ye. Mardaleyshvili and Ye. M. Volskiy, which appeared in the collection "Assimilation of Atmospheric Nitrogen by Animals and Higher Plants, published by the M. I. Volskiy laboratory (1970), essentially confirms the conclusions of Costa and Muysers. Ye. M. Volskiy was involved in a closed system, and R. Ye. Mardaleyshvili measuring ionic currents of N_2 and Ar_2 in the atmosphere, established that his test subject (Ye. M. Volskiy) produces from nitrogen-containing compounds of his body about 1.1-1.3 liters of gaseous nitrogen per hour. Hence it should be concluded that Ye. M. Volskiy loses up to 30 liters of gaseous nitrogen daily, i. e. about 35 grams of this element. Such amount of nitrogen can be obtained only through the decomposition of 220 grams of protein, i. e. at least two days of human diet. How Ye. M. Volskiy restores the protein balance of his body remains unclear.

The reasons for the catastrophic "difusion" of Ye. M. Volskiy's nitrogen /651 are not explained in the work. In our opinion phenomenon is most likely imaginary and can be attributed to errors in the method used for the study. Such an unusual phenomenon should be checked by other analytical methods. In any case the later of the examined works gives no proof of the assimilation of molecular nitrogen by the human body.

M. I. Volskiy also refers to work by Rengnault (Volskiy, 1961. p.159), which shows that marmots assimilate molecular nitrogen during hybernation. We were able to establish through diligent research that Rengnault was a french scientist (V. Regnault) who worked in the middle of the last century. His clearly obsolete data evoke doubt (Rengnault, Reiset, 1849).

There are several works (Allen, 1962; Scano, 1958; Agadzhanyan, 1967; Voronin, Polivoda, 1967), in which it is shown that in the absence of nitrogen in a gas mixture, or when it is substituted by helium, animals do not develop well. The same data are found in a collection of works of the M. I. Volskiy laboratory, published in 1970.

This is quite probable, since different gas mixtures form quite different environments for the body. These investigations, however, do not prove the assimilation of molecular nitrogen. True, Scano proves such a possibility, but he does not confirm it experimentally.

To check M. I. Volskiy's assumption of the assimilation of molecular nitrogen by mammals, A. M. Kuzin (1961), at the Institute of Biophysics of the Academy of Science of the U.S.S.R., did a twelve day test on a rat, placed in an atmosphere enriched with N_2^{15}. He demonstrated that heavy nitrogen is not included in the compounds of the body of the animal, i. e. his answer was negative.

R. Ye. Mardaleyshvili and Ye. M. Volskiy, in an article already mentioned, reported about tests with a mouse placed for one day in an atmosphere enriched with N_2^{15}. On the basis of indirect indices (determination of ionic currents) the authors conclud that the mouse assimilated about 7.5 miligram N_2^{15} per day.

It is extremely unfortunate that direct determinations of the inclusions of N_2^{15} in the body of the animal were not done, and the indirect method employed, as we showed earlier, leads to absurd conclusions.

Thus, the analyzed experiemental material absolutely is not proved.

It should be pointed out that A. A. Kudryavpsev (1961), checking the reliability of M. I. Volskiy's positions, recalculated about 300 tests with respect to the metabolism of animals, conducted in special closed chambers, and arrived at the conclusion that man and all higher animals in general are incapable of assimilating molecular nitrogen.

This all leads to the conclusion that the literature contains no confirmation of the fixation of N_2 by man or mammals.

Assimilation of Molecular Nitrogen by Bird Embryos in Tests of the M. I. Volskiy Laboratory

Most of the experimental work of the M. I. Volskiy laboratory was conducted with developing bird embryos.

M. I. Volskiy and his co-workers established that the concentration of nitrogen in the chicken eggs increases during incubation. Eleven such tests are discribed in his monograph, published in 1961.

The author supposes that the average increase in nitrogen concentration which he established in all tests, 3.2% compared with the control, is reliable and verifies the assimilation of atmospheric nitrogen during incubation. In reality, however, careful examination of all series of tests reveals a large scattering of data--the indices vary from -0.5 to +5.32%. This was seen in table I, where the data presented in M. I. Volskiy's monograph were summarized.

TABLE I. CHANGE OF NITROGEN CONCENTRATED IN INCUBATED EGGS /652

No. of tests in order	Analysis of Initial Eggs: Average nitrogen concentration in miligram per 1 gram of initial egg weight	Analysis of Initial Eggs: Limits of variation during nitrogen determinations	Analysis of Incubated Eggs: Average concentration of nitrogen in miligram per 1 gram of initial egg weight	Analysis of Incubated Eggs: Limits of variations in nitrogen determination	Established difference in nitrogen concentration
1	18.9	18.0-19.5	19.7	18.8-20.4	+4.24
2	18.7	18.0-19.2	19.3	18.4-20.6	+3.2
3	19.0	18.0-19.4	19.4	18.8-20.1	+2.1
4	18.9	18.0-19.6	19.7	17.1=21.8	+4.23
5	18.9	18.6-19.1	19.2	17.4-20.3	+1.59
6	18.8	17.6-19.6	19.4	18.9-20.7	+3.19
7	19.6	18.7-20.4	--	--	--
8	18.8	18.4-19.55	20.1	19.4-21.4	+6.91
9	18.8	18.2-19.3	19.8	17.8-20.8	+5.32
10	19.4	17.1-20.1	19.6	18.8-20.1	+1.03
11	19.2	18.0-20.6	19.1	18.0-20.4	-0.5

The average nitrogen concentration in the control of individual tests varied from 18.7 to 19.6 miligrams per gram. According to the eleven tests the control had an average of 19 miligram of nitrogen per 1 gram of material, with fluctuation of 0.9 miligrams (4.7%). Thus, the observed fixation of nitrogen (an average of 3.2%) corresponds to the level of possible variation in the protein concentration in individual series of eggs.

It is also statistical processing of the data in Table I, done by Youa Urmantsev and V. A. Pashchenko revealed that in only one third of the tests (No. 1, 6 and 9) was there any reliable addition of nitrogen.

The examined materials in general do not give sufficiently convincing proof of the assimilation of the atmospheric nitrogen during the development of chicken embryos.

We might point out that the chicken embryo consumes about 2 liters of oxygen during incubation. According to M. I. Volskiy's data an egg fixes an average of 36 miligram of N_2 during the same period, which amounts to a volume of about 30 cm^3. Thus, if we assume that N_2 is fixed by the embryo, its quantity amounts to not more than 1.5% of the oxygen consumed. What enables M. I. Volskiy to speak of a new concept of respiration?

The obvious lack of proof of previous tests prompted the M. I. Volskiy laboratory to conduct a series of additional experiments which are reported in a series of articles of the laboratory's collection, published in 1970.

We find no fundamentally new material here. In individual experiments with quail eggs during incubation the average nitrogen increments varied from 2.15 to 5.84%.

In addition, it is clear from analytical data that the averages are obtained from individual determinations which have a great scattering. Thus it follows from the data in Table II (p. 102) that the quail eggs were enriched with nitrogen during incubation (according to determination by the K'yel'dal' method) by 5.8%. This average was obtained on the basis of determinations with a scattering of 15.5% (!) (from 17.99 to 21.03 miligram of nitrogen per egg).

The data presented on page 226 (table foreign) shows that parallel samples during work by Dyum's method gives a scatter of about 15%. An average of 161 miligram of nitrogen per egg is obtained with variations of individual determinations from 149 to 174 miligrams. The K'yel'dal' method gives about 7% scatter in parallel determination.

This is quite understandable in work with heterogeneous material, but it also indicates how unconvincing are the data obtained by the M. I. Volskiy laboratory.

Given the extremely small dimensions of nitrogen fixation, reliable data can be obtained only in experiments with N_2^{15}, which incidentally, in M. I. Volskiy's opinion, is more readily assimilated by the body.

M. I. Volskiy describes in official documents a unique experiment conducted with N_2^{15}.

The description of this test shows that it was done quite incorrectly from the procedural point of view.

During the test all eggs were killed. Individual eggs perished at various stages of incubation and lay for a long period of time in a thermostat at 37-38°, subjected, of course, to the action of microorganisms. The approximate periods of deaths of the eggs were established and the content of the eggs was subjected to analysis. The experiement, so to speak, was checked with one-time repetition, i. e. only one egg was assigned to each period of analysis.

M. I. Volskiy quite seriously speaks of this experiment with "deads" as successful and producing convincing results. Analysis of the data obtained, however, produces only a negative conclusion.

Mass-spectrometric analysis, conducted by the Depatment of Chemical Kinetics, M.G.U. are not doubted, but the initial data, as already mentioned, was qualitatively low and its initial processing is not described. The Department acquired it in gilded form.

Analysis of the figures obtained, however, produces a clear mistrust of them. For the first seven days (from the 2nd through 9th days of incubation) the nitrogen increment increased by only 1%, and by 6.5% over the ensuing four days (through the 13th day) then for one day (from the 13th to 14th) it increased all of a sudden by 16.5% (!). The last period is related to the death of the last remaining live egg. Such kinetics is difficult to correlate with functional processes in a developing organism.

It must pointed out that the corresponding dynamics of increment of total and isotopic nitrogen, of which M. I. Volskiy speaks is an imaginary phenomenon, since the increment of total nitrogen was obtained through arithmetic conversion from mass-spectrometry data and not but independent chemical analysis.

Thus, the examined one-time experiment without biological doubles was carried out at the same time one questionable material and cannot be construed as proof. The above described experiment was set up in an atmosphere of oxygen with a 13.6-fold enrichment of nitrogen with the N_2^{15} isotope. /653

During our visit to the M. I. Volskiy laboratory in Gorky the co-workers of this laboratory familiarized a group of scientists from the Academy of Science of the U.S.S.R. with the results of a repeated extensive test, carried out with incubation of chicken eggs in an atmosphere enriched with N_2^{15}. In this case highly enriched nitrogen was used (up to 90 at % N_2^{15}). This test produced negative results. It was not included in the official accounts of the activity of the whole laboratory.

V. I. Kuznetsov (1970), considering the failure in his article, published in the selection "Yusvoyeniye Atmosfernogo Azota Zhivotnymi i Vysshchimi Rasteniyami", apparently substantiates the difficulty that bird eggs have in fixing N_2^{15} from the air surrounding the egg. It turns out that the eggs assimilate primarily N_2^{14}, present in the aqueous phase of the egg. Simple calculations reveal, however, that the quail egg contains only about 2.15 ml^3 gaseous nitrogen, and according to M. I. Volskiy's data about 5 or 6 ml^3 of nitrogen is assimilated during incubation. From where, then, if not from the air, does most of the fixed nitrogen come? In the last collection of works of the M. I. Volskiy laboratory " Yusvoyeniye Atmosfernogo Azota Zhivotnymi i Vysshchimi Rasteniyami (1970), it is pointed out that ionized nitrogen is more easily assimilated by living being than unionized. Ionization can produce strong changes in the state of reacting substances, and this problem requires special consideration. In particular, even weak ionization of nitrogen causes the oxides of nitrogen toform (Chevkolvich, 1971). Reacting with the lime shell of the eggs, the oxides of nitrogen can give the illusion of assimilation of elemental nitrogen by developing eggs.

Thus, the M. I. Volskiy laboratory has no convincing data about the assimilation of N_2 by bird eggs.

Information of Other Investigators on Fixation of Molecular Nitrogen by Eggs of Birds, Insects and by Other Organisms

M. I. Volskiy's test was repeated by K. A. Drel' and G. Yru. Iliyevskaya (1953). During incubation no nitrogen gain was noted in chicken eggs, nor was there any loss of nitrogen at the end of the test.

M. I. Volskiy is sharply critical of this work because according to the data of K. A. Drel' and G. Yru. Iliyevskaya the nitrogen concentration in the egg was 1.64% (16.4 miligram per 1 gram of egg weight), whereas, according to his data it should approach 19.0 miligram per 1 gram of egg weight. Hence it is concluded that the mentioned experimenters were unqualified and incompetent. This, however, would take M. I. Volskiy's data from 1962 concerning nitrogen concentration in chicken eggs we see that the average nitrogen concentration in initial chicken eggs is about 16.5 miligram of nitrogen per 1 gram of egg weight, i. e. the same as established by K. A. Drel' and G. Yru. Iliyevskaya. The question arises: Why, then, does M. I. Volskiy consider his experiments of 1962 reliable why does he not acknowledge the validity of the criticism of his work by K. A. Drel' and G. Yru. Iliyevskaya?

M. I. Volskiy made another comment on the work of K. A. Drel' and G. Yru. Iliyevskaya, a valid one this time: the incubated eggs in their tests had different weight, and M. I. Volsky chose his material more carefully. However, the analygist defect is not noted in the test by I Z. Il'yasov and R. E. Veber (1961), which M. I. Volskiy reviewed favorably, since the authors hold points of view closely aligned with his own.

To be specific can we cite the following examples. In the case of K. A. Drel' and G. Yru. Iliyevskaya the weight of the chicken eggs prior to incubation varied from 48.4 to 62.5 gram while in the experiment by I. Z. Il'yasov and R. E. Veber the weight of the pigeon eggs varied from 14.54 to 19.36 gram.

The regular increase in nitrogen in the test by I. Z. Il'yasov and R. E. Veber is noteworthy: 2.82% on the fifth day, 5.72% on the tenth, 30.18% on the fifteenth and 3.34% in the hatched pigeons.

The question involuntarily arises: Do not these figures reflect possible variations of nitrogen concentration in various batches of eggs? With fixation of N_2 the nitrogen concentration in the eggs would increase regularly with time.

In 1970, at the instruction of the presidium of the Academy of Science of the U.S.S.R., several institutes of the Academy of Science of the U.S.S.R. conducted detailed experiments to check the capacity of developing bird (quail) eggs to bond molecular nitrogen. Heavy nitrogen was used in some of these tests. The experiments yielded negative results with absolute persuasiveness[1]. Hence it should be concluded that the tests conducted by the M. I. Volskiy laboratory are not reproducable.

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[1] These materials are published in generalized form in a special article in this journal.

M. I. Volskiy asserts that A. N. Smolin and N. S. Travdin (1958), in a test conducted with silkworm pupae, established an increment of total nitrogen during the period of metamorphosis. These investigators allegedly had difficulty explaining the cause of this phenomenon. The situation is, in fact, all together different. It is shown in the cited work that glycogen is consumed faster than protein, therefore the embryo is relatively enriched with nitrogen. If, however, /654 the calculation for individuals it is easy to see that the total nitrogen concentration remains absolutely unchanged.

A. N. Smolin and N. S. Travdin write in their work: "The methods of expressing the concentration of various substances in percent of dry weight are usually employed for evaluating the relative concentration of certain substances. This method, however, may lead to erroneous conclusions when the dynamics of the process are investigated. If during some process the concentration of certain substances decreases, the relative concentration of other substances thereby increases, although perhaps no changes will occur at all."

Presented in Table II are excerpts from data of A. N. Smolin and N. S. Travdin, verifying that the calculation of nitrogen must be done individually.

TABLE II. THE CONCENTRATION OF GLYCOGEN AND NITROGEN IN SILKWORM PUPAE

Days of development	Total nitrogen	Glycogen	Days of development	Total nitrogen	Glycogen
I. Calculation by dry weight and percent			II. Calculation per individual and miligram		
1	9.4	6.2	1	103.2	50.0
17	10.4	4.9	17	101.5	15.0
20	11.0	2.1	20	103.9	1.5

It is quite obvious that M. I. Volskiy read the work he cited. Why, then, does he involve in fallacy the people who are interested in the problem that he is developing.

M. I. Volskiy generally takes many liberties with his set of so called confirmations. Thus, the works of F. V. Turchin and his co-workers (1963) on the presence of nitrogenase in leguminous plants are considered proved, although later they were subjected to investigations by A. V. Manorik (1968) and Yu. M. Loginov (1966,1969), to which M. I. Volskiy does not even refer.

M. I. Volskiy refers to letters of the frenchman M. Leveque and P. Penaud, as if substantiating his views. The cited persons asked M. I. Volskiy to forward his works and engaged with him in some discussions of general nature relative to nitrogen fixing microorganisms.

M. Leveque is a graduate student of the agricultural Institute in Marseilles. He reported to M. I. Volskiy that the isolated algae that fix molecular nitrogen, i. e. confirmed something that has been known since the beginning of this century.

P. Penaud. a businessman from Monteque, decided to produce preparations of such algae and was happy to deliver them to the requestors.

On the basis of these letters the newspaper "Sovetskaya Rossiya" published on May 22, 1970 an article (probably sponsored by M. I. Volskiy) "The Seine answers the Volga " (the french scientists thank the soviet professor). In it is stated that the discovery of the talented soviet scientist is acquiring not only recognition, but also grateful followers far from the city on the Volga. As is said in such cases, "No commentary is required here".

We do not feel it useful to analyze materials of the M. I. Volskiy laboratory related to the fixation of N_2 by plants free from microbial symbiosis. His co-worker S. V. Runkov (1968) performed some approximate tests with root tubercles and corn. These experiments are disputable on the procedural point of view, and the data obtained is so useless that it does not warrant serious discussion. Incidentally, the detailed work of Yu. M. Loginov (1969) quite clearly demonstrated that without the aid of bacteria plants cannot assimilate molecular nitrogen.

Critical review of the materials and experiments published by the M. I. Volskiy laboratory, as well as by other researchers, prompts a conclusion that man and higher organisms are incapable of assimilating molecular nitrogen in the process of their vital activity.

M. I. Volskiy's experiments with animals (bird eggs) in ordinary atmosphere turned out to be irreproducable--they yielded negative results.

The use of precise methods of analysis with stable nitrogen isotope also indicates that molecular nitrogen is not used in the vital activity of animals.

The M. I. Volskiy laboratory has no scientifically founded data on which to base the assertion that all plants are capable of fixing molecular nitrogen.

M. I. Volskiy does not make objective use of the existing literature: the /655
data accumulated by other investigators are often distorted and the material that contradicts M. I. Volskiy's views are ignored and not cited at all.

Thus, current information shows that the capacity to fix nitrogen is inherent only to lower organisms. If microorganisms exist in symbiosis with higher organisms a part of the nitrogen assimilated by the microbes may be transferred to the latter.

It should be assumed that the substitution of the nitrogen in the gaseous phase by other gases or the total exclusion of nitrogen from it has a definite effect on the organism. This is related to the change of the properties of the environment, and not to the fixation of molecular nitrogen by the organisms.

This review is based on the publications: Novaya Kontseptsiya Dykhaniya (New Concept of Respiration), by M. I. Volskiy, Gor'kiy, 1961 and the collection: Usvoyeniye Atmosfernogo Azota Zhivotnyki i Vysshimi Rasteniyami (Assimilation of Atmospheric Nitrogen by Animals and Higher Plants), Edited by M. I. Volskiy, Gor'kiy, 1970.

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